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(54) Use of beta-Glucuronidase for the treatment of immune or allergic diseases

(57) The use of beta-glucuronidase not in combination with allergens for the preparation of medicaments for the treatment of immune or allergic diseases is disclosed.

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Description

[0001] The present invention relates to the use of the beta-glucuronidase not in combination with allergens for the preparation of medicaments for the treatment of immune or allergic diseases.

[0002] Beta-glucuronidase is a enzyme capable of cleaving glucuronic groups, present in liver, spleen, tissues of the endocrine and reproductive systems of mammals and other higher animals. The main use of beta-glucuronidase is in diagnostics, for example for the determination of steroids in blood and urine. The enzyme is also widely used as reagent in immunoenzyme and molecular biology techniques.

[0003] Recently, beta-glucuronidase inhibitors have been studied as potential drugs for the therapy of inflammatory and neoplastic diseases.

[0004] The treatment of the allergic diseases is based, on the one hand, on environmental prevention and, on the other, on the use of symptomatic medicaments and/or of specific hyposensitizing immunotherapies. The latter involve the administration of the suitably formulated allergen, responsible for the disease, at regular intervals for prolonged times (3 - 5 years).

[0005] A mixture of allergens and beta-glucuronidase has been commercially available for some time, which offers some advantages compared with the administration of the allergens alone.

[0006] In particular, L. M. Mc Ewen (Brit. Med. J., 1967; 11: 509-530) treated animals, which had previously been immunized with antigen plus beta-glucuronidase or hyaluronidase, and he found that these enzymes induced hyposensitization. The same Author suggested that said activity is due to the action of the enzyme on the allergen: the mixture is prepared at the time of administration, and it decreases in time, but it can be preserved by the presence of glucose. The therapeutical action is exerted by the simultaneous administration of enzyme and allergen, and it also depends on the presence of cyclohexanediol ("the ability of beta-glucuronidase and a small dose of antigen to modify 3 diol structure appears to be optimal to control the effect of the enzyme").

[0007] Subsequently, the same Author disclosed the clinical use of a product consisting of a mixture containing the enzyme, the allergen mixture, the diol and protamine (Ann. Allergy, 1973; 31: 543-550).

[0008] A number of clinical studies has subsequently been published about the effectiveness of the mixture (Feel P. et al., 1988. Eur. J. Clin. Pharmacol., 1990, 38, 77-79; Di Stanislao C, et al. Allergie et Immunologie, 1997; 2: 39-42; Vena GA, et al., The Med. J. of Surg. and Med., 1993; 253-256; Caramia G., et al., Allergie et Immunologie, 1996; 28 : 70-73; Cantani A., et al J. Invest. Allergol. Clin. Immunol., 1996; 6(4): 270-276; Egger J. et al., The Lancet 1992; 2339: 1150-53; Astarita C. et al., J. Invest. Allergol. Clin. Immunol. 1996; 6 (4): 248-55; Ippoliti F. et al., Allergie et Immunologie, 1997; 29 : 120-5; Troise C., et al., Allergie et Immunologie, 2000; 32: 246-49).

[0009] In any event, it is evident that beta-glucuronidase has to date been used always and only as an adjuvant or enzyme activator of allergens and not due to its own immunomodulating activity.

[0010] It has now been found that beta-glucuronidase is capable of interfering in the human immune system, as it promotes the production of cytokines, in particular of IL-12, a cytokine known to be able to shift lymphocytes from TH2 (the cause of the allergic disease) to TH1 (physiological response).

[0011] Therefore, the present invention relates to pharmaceutical compositions useful in the treatment of pathologies characterized unbalanced immune system, containing beta-glucuronidase not in combination with antigens. The compositions of the invention are particularly useful in the treatment of those pathologies which can be treated or alleviated by stimulating the production of IL-12. Examples of said pathologies include all diseases which benefit from an increase in IL-12, particularly the IgE - mediated allergic ones, the most important being: asthma, rhinitis, conjunctivitis and hives.

[0012] The direct administration of IL-12 has recently been suggested as adjuvant combined with vaccines, for the treatment of viral or bacterial infections (HIV infections included), autoimmune and neoplastic diseases (WO 99/44636, WO 99/44635). The use of thalidomide to induce IL-12 production (WO 00/41547) has also been described, for the same applications.

[0013] On the other hand, Thalidomide is sadly known for its teratogenic effects, whereas the direct administration of IL-12 involves the problems connected with the use of cytokines, which are proteins difficult to dose and not free from side effects.

[0014] The use of beta-glucuronidase according to the invention is therefore advantageous and surprising, in particular compared with the above mentioned use of this enzyme in combination with the allergens, from which any activity stimulating interleukin-12 production could not be expected.

[0015] According to the invention, beta-glucuronidases of any type and origin may be used.

[0016] However, the use of a beta-glucuronidase suitably chemically modified to protect it from degradation by proteases, thereby prolonging its duration of action, is preferred. Protective techniques are well-known and comprise, for example, the introduction of polyethylene glycol residues (pegylation) or cyclohexanediol and protamine residues in the molecule.

[0017] For the envisaged for therapeutical uses, the optionally modified enzyme is suitably formulated in pharmaceutical compositions for the parenteral administration. Other administration routes, such as the oral one, can also be envisaged. Daily dosages will depend on a number of factors such as the type and severity of the disease as well as the reactivity of the immune system. As a rule, such dosages will range from about 40

U Fishman equivalents to a few µg, administered a few times at weekly or even monthly intervals.

[0018] The following example illustrates the invention in greater detail.

EXAMPLE

[0019] The activity of beta-glucuronidase on the immune system, in particular the stimulation on cytokines and specifically on interleukin 12, alpha-TNF, alpha-interferon and IL-10, was assayed. The test can be summarized as follows: CMNs were cultured in 24-wells plates ($2x10^6$ cells/ml) in complete medium for 4 hours at 37°C under humidified atmosphere enriched in 5% CO_2. Wells were washed 3 times with PBS, pH 7.2, the adhered cells were treated with the specific stimulus (beta-glucuronidase) and after a 3 day incubation supernatant was taken, aliquoted and stored at -20°C until use. The amount of IL-12 was determined on each sample. The IL-12 contained in the supernatant was quantitized by ELISA (Endogen, Wobum, MA, USA).

[0020] The results reported in the annexed figure evidence that treatment with beta-glucuronidase stimulates IL-12 production.

Claims

1. The use of beta-glucuronidase not in combination with allergens for the preparation of medicaments for the treatment of immune or allergic diseases.

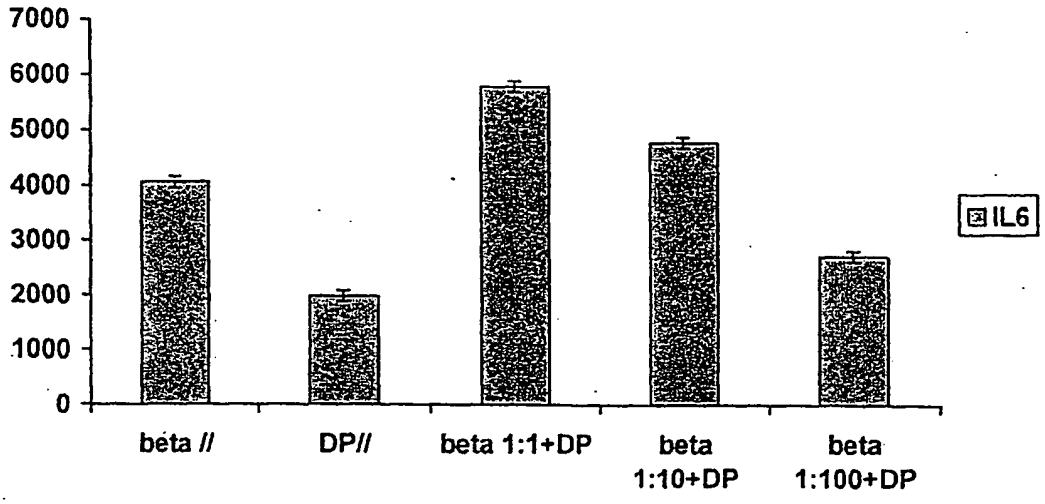
2. The use as claimed in claim 1 for the treatment of those diseases which can be treated or alleviated by a stimulation of the production of cytokines, particularly of IL-12.

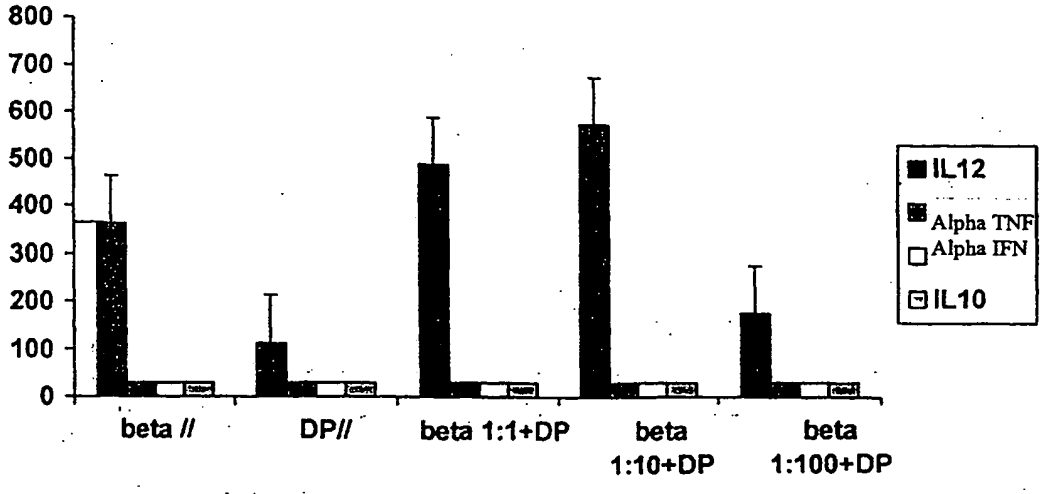
3. The use as claimed in claim 1 or 2 for the treatment of all of the diseases which benefit from an increase in IL-12.

4. The use as claimed in claim 3 for the treatment of IgE-mediated diseases.

5. The use as claimed in claim 4 for the treatment of asthma, rhinitis, conjunctivitis and hives.

FIGURE





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EUROPEAN SEARCH REPORT

Application Number
EP 01 83 0058

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 99 48478 A (YACOBY ZEEVI ORON ;FRIEDMAN MARK M (IL); INSIGHT STRATEGY & MARKET) 30 September 1999 (1999-09-30) * the whole document *	1-5	A61K38/47 A61P37/00
X	DE 28 40 173 B (KÖHLER VALENTIN) 3 January 1980 (1980-01-03) the wholed document especially example 4	1-5	
X	GB 1 179 787 A (POPPER FRANTISEK; GOTTFRIED SIEGFRIED) 28 January 1970 (1970-01-28) * the whole document *	1-5	
X	GB 2 205 746 A (MCEWAN LEONARD MAITLAND) 21 December 1988 (1988-12-21) the whole document especially examples 3 and 4	1	
A	DI STANISLAO C ET AL: "A double-blind, placebo-controlled study of preventive immunotherapy with E.P.D., in the treatment of seasonal allergic disease." ALLERGIE ET IMMUNOLOGIE, (1997 FEB) 29 (2) 39-42, XP001024862 * the whole document *	1-5	TECHNICAL FIELDS SEARCHED (Int.Cl.7) A61K C12N
A	US 5 674 483 A (IRVIN CHARLES G ET AL) 7 October 1997 (1997-10-07) * the whole document *		

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	5 October 2001	Stein, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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05-10-2001

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 9948478	A	30-09-1999	US	6153187 A	28-11-2000
			AU	3107799 A	18-10-1999
			WO	9948478 A1	30-09-1999
			US	2001006630 A1	05-07-2001
DE 2840173	B	03-01-1980	DE	2840173 B1	03-01-1980
GB 1179787	A	28-01-1970	DE	1767038 A1	09-09-1971
			FR	8032 M	03-08-1970
GB 2205746	A	21-12-1988	IT	1218080 B	12-04-1990
US 5674483	A	07-10-1997	US	5908620 A	01-06-1999
			US	5906815 A	25-05-1999

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82